

Means of tumor therapy

Description

5 The invention in question relates to a pharmaceutical agent on the basis of a combination of anti-oestrogen, alkylphospholipids and phospholipids, its manufacture and use. Fields of application of the invention are medicine and the pharmaceutical industry. In medicamentous tumor therapy, optimal treatment is repeatedly inhibited by the occurrence of resistance against the pharmacon and by toxic side-effects. A part of these undesired effects can be cancelled or soothed by encapsulation of the medicaments in liposomes (D. D. Lasic and D. Papahadjopoulos, Medical Applications of Liposomes, Elsevier, 1998). Liposomal anthracyclins have reached the stage of extended clinical application. Specific benefits result if phospholipids with an inherent anti-tumor effect are used to form the liposomes, e.g. alkyl phospholipids (Arndt et al. Drugs of Today 1998, 34, 83-96).

Alkyl phospholipids are a new type of compound, the effect of which against tumor growth is achieved by effects on the cell membrane (Alkylphosphocholines: An update, Drugs of Today, Vol. 34, Suppl. F, 1998).
20 Under certain conditions, alkylphospholipids result in supra-molecular structures, inter alia liposomes, with more favourable properties compared with the monomeric or micellar organized compound (DE 41 32 345 A1, DE 44 08 011 C1). Further substances with anti-neoplastic effect can be included in these liposomes with an inherent anti-tumor effect (Arndt et al., Breast Cancer Res. Treatm. 43 (1997) 237-246, DE 44 08 011 C1).
25

Mamma carcinomas, the most frequent tumor in women, can be influenced in about 75% of the cases by endocrine measures. Competitive hormone therapy by means of Tamoxifen is of particular importance in this context; in it, the endogenous hormones are antagonised at the receptor.

5

Treatment with Tamoxifen, which is low in side-effects, is however limited by development of resistance against the pharmacon. The causes of the resistance are, inter alia, alterations of the ligand and its binding to the oestrogen receptor (ER), loss or alteration of the ER, alterations of transcription factors or the ER-associated protein or blockage through anti-oestrogen binding proteins (Katzenellenbogen et al., Breast Cancer Res. Treat. 44 (1997) 23-38; Osborne, New Engl. J. Med. 339 (1998) 1609-18; US005904930A).

The objective of the invention is the creation of a medication formulation on the basis of anti-oestrogen, alkylphospholipid and phospholipids, which is effective in anti-oestrogen resistant tumors and which minimises or prevents the development of resistance.

The invention is characterised by the primary claims, the sub-claims being preferred variants.

20

The essential feature of the invention is the combination of alkylphospholipid with an anti-neoplastic effect and an anti-oestrogen in a lipid

vesicle. A preferred example is octadecyl-(N,N-dimethylpiperidin-4-yl)-phosphate (OPP), Tamoxifen (Tam) in phosphocholine (PC) vesicles.

In detail, the agent according to the invention is characterised by the following composition:

- an alkylphospholipid (with anti-neoplastic effectivity)
- a water or lipid-soluble anti-oestrogen with anti-neoplastic effectivity
- an anti-neoplastically inert phospholipid
- if need be, cholesterol or any other suitable sterol
- if need be, a lipid with positive or negative surface charge
- if need be, a polyethylene glycol modified lipid (PEG lipid)
- if need be, further active agents and pharmaceutically customary carrier and ancillary materials.

Alkylphospholipids with an anti-tumor effect of general structure I are used as phospholipid analogs.

Structure I:

R-Y-P-X

This formula contains the following meanings:

R: an alkyl, alkenyl or alkynyl residue with 12 to 22 C atoms

Y: oxygen, sulphur or CH₂

P: phosphate group (PO₂)

X: a choline or modified choline rest or serine,
ethanolamine, glycerine groups or synthetic
modifications of these groups such as the piperidine-4-yl
group

5

Suitable compounds are hexadecylphosphocholine,
octadecylphosphocholine, erucyl- phosphocholine, octadecyl-[2-(N-
methylpiperidinio)ethyl]-phosphate, octadecylphospho-ethanolamine and
hexadecylphosphoserine.

The water or lipid-soluble anti-oestrogen associated with the phospholipid
analogs is represented by Tamoxifen, Droloxifene, Toremifene, Idoxifene,
Raloxifene, Miproxifene-Phospat (TAT-59), ICI 1643,384, ICI 182,780 and the
main metabolites of Tamoxifen, 4-hydroxytamoxifen and N-desmethyldtamoxifen.

Phospholipids without their own anti-neoplastic effect are lipids from
natural sources or of synthetic origin such as customarily used for liposome
production, e.g. phosphatidylcholine.

20

Suitably, polyethylene glycol modified phosphatidylethanolamine in the
molecular weight range of 1000 - 6000 Dalton is used as a PEG lipid. Inter alia,
1,2-Distearoyl-s,n-glycero-3-phosphoethanolamine-N-polyethylenglycol,

MG2700; (PEG₂₀₀₀DSPE) and 1,2-Dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-polyethylenglycol, MG5750 (PEG₅₀₀₀DPPE) are suited.

5 The use of compounds which are simultaneously a PEG lipid and an anti-neoplastically effective phospholipid analog is also beneficial, for example hexadecylphosphoethanolamine-N-polyethylenglycol.

According to the invention, an anti-neoplastically inert lipid of a natural or synthetic origin is suitably used as a base lipid for the membrane formation, such as phosphocholine, serine, ethanolamine, glycerol or other similar lipids, with the ratio of lipid to anti-oestrogen being 0-10 : 1 (mass ratio m/m). Suitably, cholesterol or another suitable sterol such as sitosterol is contained, with the sterol being in a mol ratio of 0-1 : 1 to the alkylphospholipid. The liposomal form suitably comprises single-layered or multi-layered vesicles or the liposomes are available as "reverse evaporation vesicles".

10
15
20 The effect of the agent of overcoming resistance according to the invention can be proven both *in vitro* and *in vivo*. The means of tumor therapy according to the invention is pharmaceutically stable, physiologically outstandingly tolerable and particularly suitable for intravenous application. Undesired metabolism of the anti-oestrogens is avoided or reduced, improved resorption and distribution of the pharmacon is achieved. Anti-oestrogens difficult to dissolve in water can

well be applied in a liposomal form. The means is therefore outstandingly suited for application in tumor therapy.

The invention is explained by the following examples:

Example 1:

4.62 mg octadecyl-(1,1-dimethyl-piperidino-4-yl)-phosphate (OPP; 10 μ mol), 0.387 mg Z-4-hydroxy-Tamoxifen (HO-Tam, 1 μ mol), 1.55 mg cholesterol (4 μ mol), and 1.1 mg dicetylphosphate (DCP; 2 μ mol) are completely dissolved in 25 ml chloroform/methanol (7/3; v/v) and the solvent then completely evaporated on a rotation evaporator. The finely distributed lipid film gained is re-suspended with 1 ml of phosphate-buffered salt solution (PBS, pH 7.4) and intensively moved for at least 3 hours at room temperature on a vibration machine following addition of some glass pearls. The suspension of multi-layered vesicles (MLV) obtained is then repeatedly extruded through polycarbonate filters, pore diameter 100 nm, with a LiposoFast basic system (Avestin, Inc. Ottawa, Canada) until vesicles with an average diameter around 100 nm with a unimodal distribution of sizes and a polydispersity index of less than 0.2 (Dynamic Light Scatter Measurement, DLS) are obtained.

The content of OPP, HO-Tam, CH and DCP is checked by means of HPTLC. Above 85 % of the original amount is retained. The composition of the

liposomes is unchanged compared with the original composition (deviation < 5%). These HO-Tam liposomes are suitably used for *in vitro* examinations.

Example 2:

36 mg OPP, 72 mg Tamoxifen citrate (Tam), 144 mg phosphatidylcholine (PC) and 8.5 mg DCP are completely dissolved in 100 ml chloroform/methanol (7/3; v/v) and the solvent then completely evaporated on a rotation evaporator. The finely distributed lipid film gained re-suspended with 12 ml of citric acid/phosphate buffer (pH 6.08), and intensively moved for at least 3 hours at room temperature on a vibration machine following addition of some glass pearls. An MLV suspension is obtained, which is heterogeneous in its size composition with vesicle diameters of between 100 and 5000 nm.

These Tam liposomes are suitably used for *in vitro* examinations and as initial liposomes for vesicles of a defined size.

Example 3

36 mg OPP, 72 mg Tamoxifen citrate (Tam), 144 mg phosphatidylcholine (PC) and 8.5 mg DCP and additionally 9.7 mg N-(O-methyl-polyethylenglycyl)-1,2-distearyl-s,n-glycero-3- phosphoethanolamine (PEG₂₀₀₀DSPE) are completely dissolved in 100 ml chloroform/methanol (7/3; v/v) and the solvent then completely evaporated on a rotation evaporator. The finely distributed lipid film gained is re-suspended with 12 ml of citric acid/phosphate buffer (pH 6.08) and

intensively moved for at least 3 hours at room temperature on a vibration machine following addition of some glass pearls. An MLV suspension is obtained, which is heterogeneous in its size composition with vesicle diameters of between 100 and 5000 nm. These Tam liposomes are suitably used for *in vitro* examinations and as initial liposomes for vesicles of a defined composition.

Example 4:

Tam MLV's from example 2 are repeatedly extruded through polycarbonate filters, pore diameter 200 nm, with a LiposoFast basic system (Avestin, Inc. Ottawa, Canada) until a unimodal size distribution around 180 nm is achieved with a poly-dispersity index of less than 0.35 (Dynamic Light Scatter Measurement, DLS).

The content of OPP, Tam, CH and DCP is checked by means of HPTLC. A liposome suspension containing about 75 % of used Tam and 98 % of OPP is obtained. In addition, the composition of the liposomes is unchanged compared with the original composition (deviation < 5%). These Tam liposomes are suitably used for *in vivo* examinations.

Example 5:

Peg-Tam MLV's from example 3 are repeatedly extruded through polycarbonate filters, pore diameter 200 nm, with a LiposoFast basic system

(Avestin, Inc. Ottawa, Canada) until a unimodal size distribution around 185 nm is achieved with a poly-dispersity index of less than 0.33 (Dynamic Light Scatter Measurement, DLS). The content of OPP, Tam, DCP und Peg₂₀₀₀DSPE is checked by means of HPTLC. A liposome suspension containing about 75 % of used Tam and 98 % of OPP is obtained. In addition, the composition of the liposomes is unchanged compared with the original composition (deviation < 5%). The Peg-Tam liposomes are suitably used for *in vivo* examinations.

Example 6:

HO-Tam liposomes from example 1 are diluted with RPMI medium with 10% foetal calves' serum (without added indicator, with adriamycin/streptomycin) in such a way that a concentration of 200 nmol/ml of OPP is reached, then being further serially diluted down to 0.78 nmol/ml. The concentration of HO-Tam active agent is then accordingly 20 nmol/ml to 0.08 nmol/ml.

The breast cancer cells MCF7, which are sensitive towards Tamoxifen, and MCF7-R, which are resistant to anti-oestrogen, are seeded into 96-well plates with a density of 2×10^4 cells/well and incubated on the following day with HO-Tam liposomes, control liposomes of the composition of the HO-Tam liposomes, but without HO-Tam, HO-Tam, dissolved in DMSO and DMSO of the same amount as needed to dissolve the HO-Tam, for three days. After this, the supernatants are removed, the cells washed with PBS and then the cell growth

inhibition determined with the MTT assay. For this, the cells are incubated with 200 µl MTT solution (4,6-dimethylthiozol-2-yl-2,5-diphenyl-tetrazolium; 0.5 mg/ml) for 4 hours at 37°C, 170 µl of the supernatant carefully removed and the precipitated formazan crystals completely dissolved with a 70% Isopropyl alcohol solution by intensive pipetting and shaking. After this, the 96-well plates are photospectroscopically measured at 540 nm and the growth inhibition calculated in comparison to the growth of untreated cells. A growth inhibition as portrayed in Figure 1 is obtained.

Example 7:

Tam liposomes according to Example 4 are used for the *in vivo* test. As a tumor model, breast cancer 3366/Tam is transplanted onto female NMRI nude mice and the treatment started when the tumor is palpable. The animals are given one dose of liposomes with 50 mg/kg Tam (and correspondingly 25 mg/kg OPP) twice a day for 4 weeks. As controls, liposomes containing no Tam are administered, in addition one group being treated with free Tam. The tumor growth in relation to the control group (physiological salt solution) is determined and portrayed as a percentage T/C figure in Table 1.

Table 1:

Therapeutic effectivity of Tamoxifen liposomes compared with the resistant breast cancer tumor 3366/Tam

Group	Substance	Dose, Tam/Lipid	Alteration of T/C body weight	
		mg/kg/injection	% (day 29/51)	%
A	Solvent		3	
B	Tamoxifen	50/0	-5	91
C	Tamoxifen liposomes	50/25	-5	63*
D	Control liposomes	0/25	-4	88

* Significantly different from Tamoxifen and the solvent control ($p < 0.05$)